Equivalent performance of Xuri Cell Expansion System W25 and WAVE Bioreactor System 2/10 for the culture of T lymphocytes

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Application note 29-0929-38 AA

Cell expansion

Equivalent performance of Xuri™ Cell Expansion System W25 and WAVE Bioreactor™ System 2/10 for the culture of T lymphocytes

This Application note describes a robust process for manufacturing human T cells using WAVE Bioreactor System 2/10 and Xuri Cell Expansion System W25. Data presented here demonstrate that the growth rate of T cells on both systems were comparable. In addition, the expanded T cells were phenotypically similar in terms of their differentiation state, and were biologically functional, as demonstrated by their ability to secrete a predominantly Th1 cytokine milieu.

Introduction

WAVE Bioreactor System 2/10 (WAVE 2/10) is an established platform for the manufacture of clinical grade T cells. Cells are grown in a single, disposable Cellbag™ bioreactor in a functionally closed, regulated environment. High cell densities are reached by combining the gas mixing properties of the rocking base with media perfusion to maintain nutrient and waste metabolites. As such, the generation of 2×10^{10} cells/l of media are readily achieved.

Xuri Cell Expansion System W25 (Xuri W25) is the latest innovation from GE Healthcare Life Sciences for expansion of human suspension cells (Fig 1). It represents the next generation of bioreactors and is designed for progressing cell manufacture from a research environment to a regulated, commercial environment. Its features include advanced process control, remote monitoring and the ability to operate multiple units from a single workstation.

To demonstrate that the quantity and quality of T cells produced using Xuri W25 are at least equivalent to WAVE Bioreactor System 2/10, T cells were cultured on both systems in parallel.

Materials and methods

Activation of T cells in static culture

Frozen human peripheral blood mononuclear cells (PBMCs) were thawed, washed twice, and cultured in T225 flasks

Fig 1. Human T cells were grown on WAVE Bioreactor System 2/10 (left) and Xuri Cell Expansion System W25 (right) and the relative cell-culture performance of the two instruments was evaluated.

at 1×10^6 cells/ml in X-Vivo™-10 (Lonza) supplemented with 5% heat-inactivated human serum (GE Healthcare), 2 mM GlutaMAX™ (Invitrogen), 1% penicillin-streptomycin (Invitrogen) and 20 ng/ml of IL-2 (Peprotech). T cell expander CD3/CD28 beads (Invitrogen) were added to the culture at a ratio of 3:1 beads:$CD3^+$ T cell. After 3 d, cells were counted and maintained at 0.5×10^6 cells/ml for an additional 2 d.

Expansion of T cells in Cellbag bioreactors

After the 5 d in static culture, cells were transferred to two Cellbag 2L bioreactors. One Cellbag was loaded onto the WAVE Bioreactor 2/10 and the other was loaded onto the Xuri W25. Both WAVE 2/10 and Xuri W25 systems were set at 37°C with a rocking rate of 10 rpm and a rocking angle of 6°. Cells were maintained at 0.5×10^6 cells/ml by adding media until the maximum volume of 1000 ml per Cellbag was reached. Once cell concentrations had reached a minimum of 2×10^6 cells/ml media, perfusion commenced. Two comparative experiments, denoted A and B, were performed, each comparing WAVE 2/10 and Xuri W25 using the perfusion rates shown in Table 1.

Table 1. Perfusion rates in two experiments (A and B) of T cell culture in Cellbag bioreactor 2 L on WAVE 2/10 and Xuri W25 systems

	Perfusion rate (ml/24 h)	
Day of culture	Experiment A	Experiment B
7	500	500
8	750	500
9	750	500

The perfusion rates were set to ensure that ammonium levels were maintained below 2.0 mmol/l, lactate levels were maintained below 2.0 g/l, and glucose levels were above 1.0 g/l.

The media perfusion program on WAVE 2/10 is run as semi-continuous, with shot volumes of 50 ml evenly spaced throughout a 24 h period. For example, a perfusion rate of 500 ml/24 h equates to the removal and replacement of 50 ml approximately every 144 min (or 10 times throughout the 24 h period). Xuri W25 is designed to routinely run perfusion as a continuous process. As the purpose of the study was to demonstrate equivalent performance of the two bioreactor systems, the semi-continuous perfusion was replicated on the Xuri W25, by writing a program in the Method Editor module of Xuri W25 UNICORN™ software (Fig 2). This program ensured that both bioreactor systems were perfusing media at the same rate and in the same manner.

```
0.00  Base: Time
0.00  Phase: Method Settings

0.00  Base: SameAsMain
0.00  Loop: 9999, feed shot_1

0.00  Base: SameAsMain
2.40  Start harvest pump: Continuous, 60 {sec}, 50.0 {RPM}
2.40  Hold until: Weight, Less than, 0.954 {kg}, 5.00 {base}
2.40  Stop harvest pump
2.40  Start feed pump: Continuous, 60 {sec}, 50.0 {RPM}
2.40  Hold until: Weight, Greater than, 0.996 {kg}, 5.00 {base}
2.40  Stop feed pump
2.40  End Loop
0.00  End_Block
```

Fig 2. The program in the Method Editor module of UNICORN software written to run the semi-continuous perfusion schedule on Xuri W25. This script was used to ensure that the perfusion regimes on WAVE 2/10 and Xuri W25 systems were equivalent. The script represents a perfusion rate of 500 ml/24 h. To change the perfusion rate, the wait period between shots on the script was adjusted.

Phenotypic analysis

T cells were phenotyped by flow cytometric analysis at days 0 and 10 of culture: 1 × 10^6 cells were stained with CD3-PerCP™Cy™5.5, CD4-PE, CD8-Alexa Fluor™ 488, CD28-APC, CD27-V450, or CD57-APC. The stained cells were analyzed on FACSFortessa flow cytometer using FACSDiva™ software, according to the manufacturer's instructions (reagents, instrument and software from BD™ Biosciences).

T cell reactivation and cytokine production

On day 10 of culture, cells were harvested from the Cellbag bioreactors and reseeded in 2 ml of fresh T cell media at 1 ×10^6 cells/ml in a 24-well plate. The cells were reactivated with CD3/CD28 Dynabeads™ for 18 to 20 h. The supernatants were collected and measured for the production of 7 different cytokines (Millipore™ multiplex assays).

Results

T cells were concurrently expanded using WAVE Bioreactor System 2/10 and Xuri Cell Expansion System W25 to determine whether comparable numbers of expanded cells could be achieved. The cumulative cell growth in a Cellbag using the systems is presented in Figure 3. Similar viable cell numbers were reached in both WAVE 2/10 and Xuri W25 systems. The cumulative fold expansion growth of peripheral blood T cells was 272-fold for WAVE 2/10 and 285-fold for Xuri W25 (average of experiment A and B), with viabilities above 90% (results not shown).

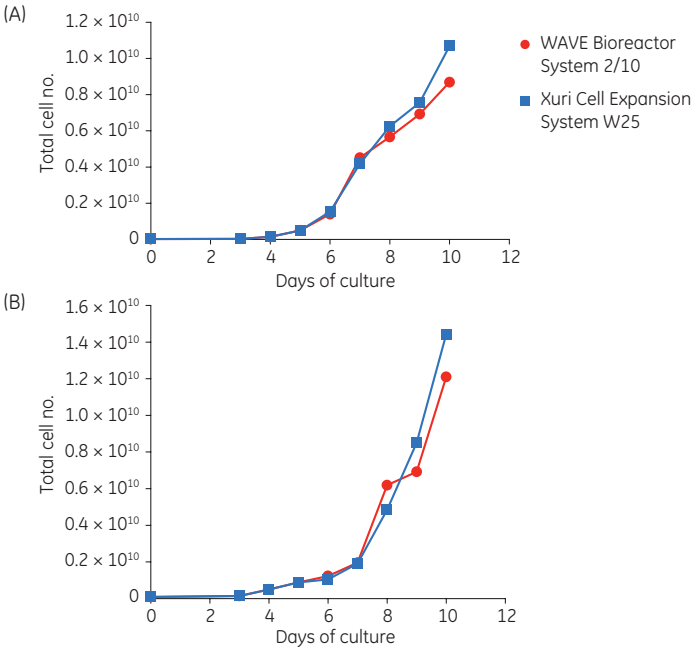

Fig 3. Growth of cells cultured on either WAVE 2/10 (circles) or Xuri W25 (squares) systems in experiments A and B at the perfusion rates shown in Table 1.

To determine whether the phenotypic composition of the expanded T cells in both systems was similar, the expression patterns of several cell surface markers were compared by flow cytometry. The expression pattern of the cell surface markers CD27 and CD28 allows the differentiation state of T cells to be assessed. Naïve and early activated cells are $CD27^+CD28^+$, effector T cells are $CD27^-CD28^+$, and late effectors or "aged" T cells are $CD27^-CD28^-$. The expression of these two markers on cells grown on WAVE 2/10 and Xuri W25 was equivalent (Fig 4) and after 10 d of culture, the majority of cells were in the early/intermediate stages of differentiation.

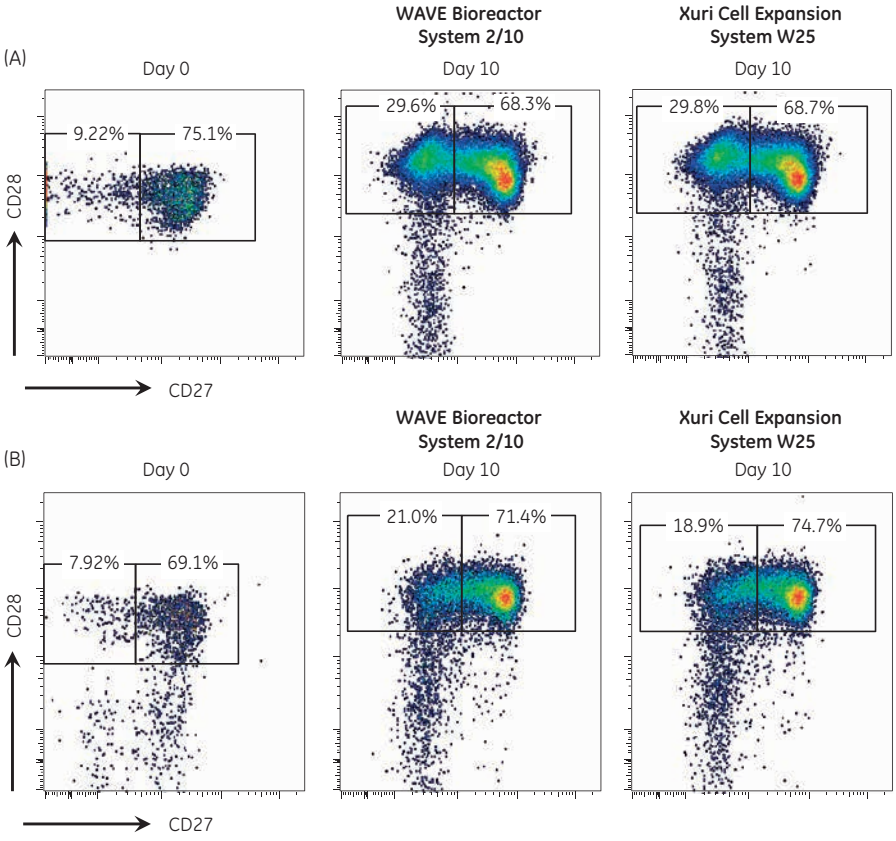

Fig 4. Expression of costimulatory molecules CD28 and CD27 in $CD3^+$ T cells using WAVE 2/10 and Xuri W25 systems. Representative dot plots are shown at day 0 and at day 10 of culture. Lymphocytes were gated based on their forward- and side-scattter profile. (A) Percentage $CD28^+CD27^+$ within $CD3^+CD4^+$ T cells. (B) Percentage $CD28^+CD27^+$ within $CD3^+CD8^+$ T cells.

Continuous activation and proliferation can drive T cells to a state of senescence, which can be detected by the expression of the cell surface marker CD57. Analysis of CD57 expression showed that there was no difference between cells that had been grown on WAVE 2/10 and Xuri W25 systems, nor was there any accumulation of $CD57^+$ T cells throughout the 10 d culture period (Fig 5).

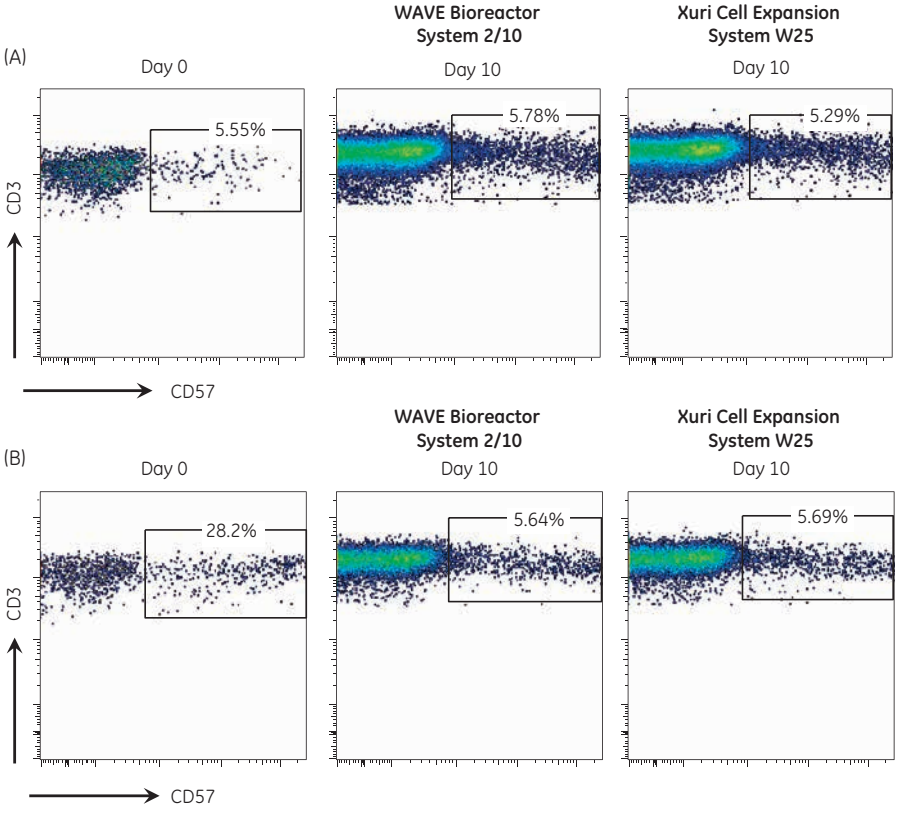

Fig 5. Expression of CD57 in $CD4^+$ and $CD8^+$ T cells using WAVE 2/10 and Xuri W25 systems. Representative dot plots are shown at day 0 and at day 10 of culture. (A) Percentage $CD57^+$ within $CD3^+CD4^+$ T cells. (B) Percentage $CD57^+$ within $CD3^+CD8^+$ T cells.

To ensure that the expanded cells were still biologically functional at the end of the 10 d culture period, the cells were reactivated with CD3/CD28 beads for 18 to 20 h and cytokine production was measured. The secretion of both Th1 (IFNγ, GM-CSF, TNFα, and IL-2) and Th2 (IL-4, IL-5, and IL-10) cytokines was analyzed. There was no difference in the amounts of cytokine secreted when comparing cultures from WAVE 2/10 and Xuri W25. It was also noted that a Th1 cytokine profile was dominant, which favors a cell-mediated immune response *in vivo* (Fig 6).

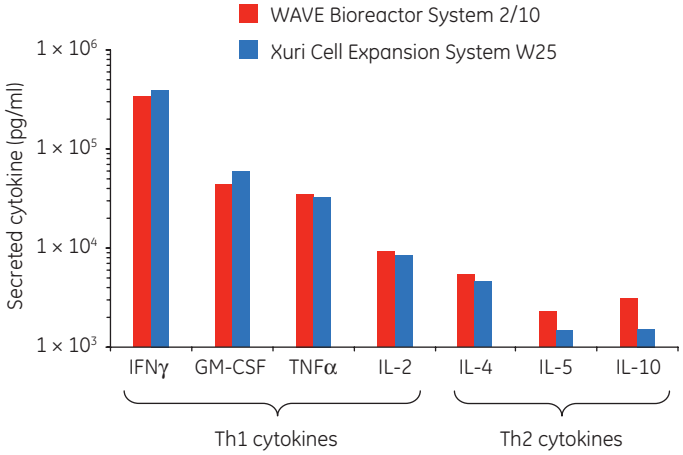

Fig 6. Concentration of soluble cytokine after reactivation of T cells. On day 10 of culture, cells cultured using using WAVE 2/10 and Xuri W25 systems were reactivated with CD3/CD28 beads for 18 to 20 h and cytokine secretion was measured. Cytokine concentration is in pg/ml.

Conclusion

This study demonstrates that both WAVE Bioreactor system 2/10 and Xuri Cell Expansion System W25 are able to support the rapid expansion of primary human T cells. No differences in cell number, quality, or function were observed between T cells grown on WAVE 2/10 compared to the Xuri W25. In addition, Xuri W25 offers benefits that facilitate the progress of cell manufacture from a research environment to a regulated, commercial environment. Full details can be found in reference 1.

References

1. Data file: Xuri Cell Expansion System W25, GE Healthcare, 29-0618-52, Edition AC (2013).

Ordering information

Product	Code number
Xuri Cell Expansion System W25, Base Unit	29-0645-68
Xuri Cell Expansion System W25, Cellbag Control Unit Full: Gas flow/mix, pH, and DO	29-0646-02
Xuri Cell Expansion System W25 Pump	29-0645-71
UNICORN 6.3.2 Workstation Package, software and license	29-0469-18
WAVE Bioreactor 2/10 System, BASE2/10EH	28-4115-00
WAVE Bioreactor 2/10 system Perfusion Controller	28-9875-84
Cellbag 2 L, BC10 Perf DO	28-9376-52

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com/xuri

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

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